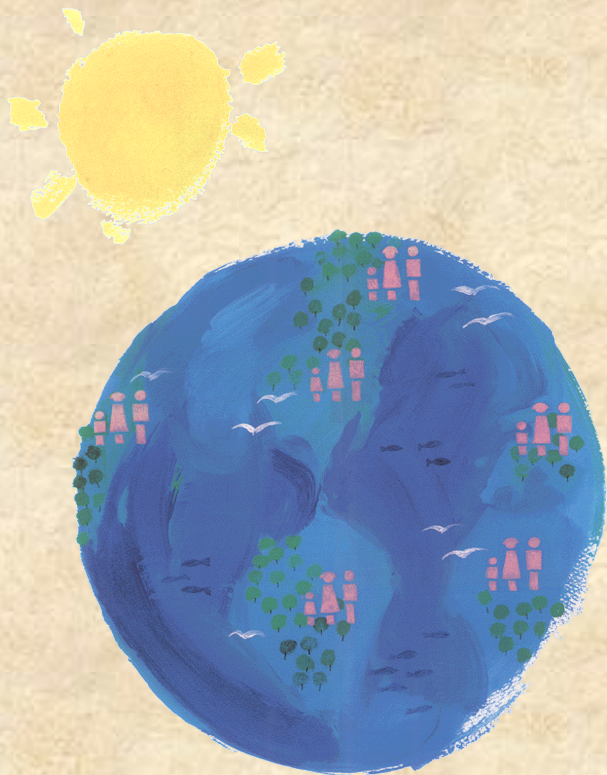


NICEATM

National Toxicology Program Interagency
Center for the Evaluation Of Alternative
Toxicological Methods

ICCVAM

Interagency Coordinating Committee
on the Validation of Alternative
Methods



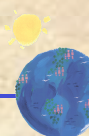
The Isolated Chicken Eye (ICE) Test Method

BRD Summary

Expert Panel Meeting
January 11-12, 2005
Bethesda, Maryland

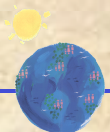


ICCVAM
NICEATM



Current U.S. Regulatory Status of ICE

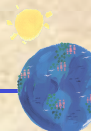
- ICCVAM agencies were surveyed and, to the best of their knowledge, ICE test method data have not been submitted to U.S. Regulatory Agencies.



Primary ICE Data Sources

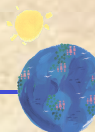
Study		Accuracy			Intralab		Interlab	
		GHS	EPA	EU	CVs	GHS classific.	CVs	GHS classific.
Prinsen and Koëter (1993)	S	3	3	8	-	-	-	-
	NS	7	7	13	-	-	-	-
	Total	10	10	21	-	-	-	-
Balls et al. (1995)	S	22	20	21	-	-	59	22
	NS	34	34	38	-	-		34
	Total	56	54	59	-	-		56
Prinsen (1996)	S	0	0	6	-	-	-	-
	NS	29	29	38	-	-	-	-
	Total	29	29	44	-	-	-	-

S: severe or corrosive irritants; NS: nonsevere irritants or nonirritants; classific.: classification; CV: coefficient of variation



Other ICE Reports Considered

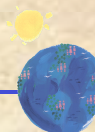
- Two other reports were identified that could not be used for an evaluation of accuracy or reliability due to the lack of:
 - comparative *in vivo* rabbit test data
 - incomplete substance identification
 - quantitative *in vitro* data
- These reports discussed in Section 9
- No additional data were obtained



ICE Database

- 121 Different substances evaluated in three tests
- 15 Chemical classes tested*
 - Most frequent classes:
 - alcohols
 - acids
 - surfactants
- 14 Product classes tested*
 - Most frequent classes:
 - chemical/pharmaceutical intermediates
 - herbicides/pesticides
 - industrial chemicals
 - soaps/surfactants/detergents

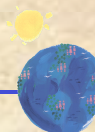
* Classes with at least 3 entries



Major ICE Protocol Variations

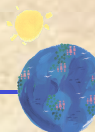
Study	n	# Eyes			Exp. Dur.	CO	CS	FR	Morph. Eval.	Histopath.
		Neg	Treat	Pos						
Prinsen and Koëter (1993)	21	1	5	-	10 sec	X	X	X	X	Case-by-case basis
Balls et al. (1995)	59	1	3	-	10 sec	X	X	X	X	Not specified
Prinsen (1996)	44	1	3	-	10 sec	X	X	X	X	Not specified

CO: Corneal opacity; CS: Corneal swelling; Exp. Dur.: Exposure duration; FR: Fluorescein retention; Histopath.: Histopathology; Morph. Eval.: Morphological evaluation; n: Number of substances tested; Neg: negative control; Pos: positive control; Treat: test substance treated



ICE Accuracy

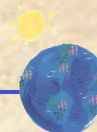
- Ability to correctly identify ocular corrosives and severe irritants determined for
 - GHS classification system (Category 1)
 - EPA classification system (Category I)
 - EU classification system (R41)
- Accuracy statistics calculated:
 - for each ICE test method protocol by report and where appropriate
 - classifications were pooled into one classification per substance (i.e., majority call among studies used)
 - using individual studies, where a balanced design existed (multiple substances in multiple labs)



Recommended ICE Version Accuracy

Statistic	GHS (n=92)*		EPA (n=90)*		EU (n=121)*	
	%	n	%	n	%	n
Accuracy	82	75/92	82	74/90	85	103/121
Sensitivity	60	15/25	61	14/23	70	26/37
Specificity	90	60/67	90	60/67	92	77/84
False Positive Rate	10	7/67	10	7/67	8	7/84
False Negative Rate	40	10/25	39	9/23	30	11/37

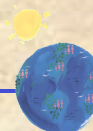
*from Prinsen and Koëter (1993), Balls et al. (1995), and Prinsen (1996);
Additional chemicals available for EU analysis only (individual animal data not available for GHS or EU classification)



ICE GHS Accuracy By Chemical/Physical Class (1)

Class	# of Substances			False Positive Rate		False Negative Rate	
	Total	Cat 1	Cat 2A, 2B, NI	%	n	%	n
OVERALL	92	25	67	10	7/67	40	10/25
Surfactant	13	7	6	0	0/6	57	4/7
Alcohol	10	0	10	50	5/10	-	-
Acid	7	5	2	0	0/2	20	1/5
Acetate	6	0	6	17	1/6	-	-
Heterocyclic	6	5	1	0	0/1	40	2/5
Hydrocarbon	6	2	4	0	0/4	50	1/2

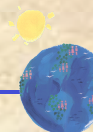
•Prinsen and Koëter (1993), Balls et al. (1995), and Prinsen (1996)



ICE GHS Accuracy By Chemical/Physical Class (2)

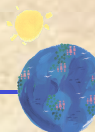
Class	# of Substances			False Positive Rate		False Negative Rate	
	Total	Cat 1	Cat 2A, 2B, NI	%	n	%	n
Inorganic	3	1	2	0	0/2	100	1/1
Ketone	3	0	3	33	1/3	-	-
Amine	2	1	1	0	0/1	0	0/1
Acyl halide; Lactone; Aldehyde; Amide; Organometallic; Organophosphate	1	0	1	0	0/1	-	-
Alkali	1	1	0	-	-	0	0/1
Diol	1	1	0	-	-	100	1/1
Solids	23	11	12	0	0/12	55	6/11
Liquids	69	14	55	13	7/55	29	4/14

•Prinsen and Koëter (1993), Balls et al. (1995), and Prinsen (1996)



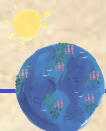
Limitations of ICE Accuracy Analysis

- Lack of individual rabbit data for all substances prevents an accuracy evaluation using the GHS and EPA classification schemes.
- The small number of substances in each chemical class allow for limited conclusions with respect to the accuracy of ICE by chemical class or physicochemical property. However, it appears that:
 - Alcohols tend to be overpredicted
 - Surfactants and solids tend to be underpredicted





ICE Reliability

- **Intralaboratory Repeatability and Reproducibility**
 - Not conducted due to the lack of published intralaboratory ICE data
- **Interlaboratory Reproducibility**
 - Qualitative analysis: Extent of agreement between testing laboratories when identifying ocular corrosives and severe irritants
 - Quantitative analysis: Coefficient of variation

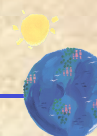


ICE Classification Agreement Among Four Laboratories

% Interlaboratory Agreement	GHS (59 substances)*		EPA (59 substances)*		EU (59 substances)*	
	%	n	%	n	%	n
100% (all)	75	44/59	75	44/59	76	45/59
 75% (all)	90	53/59	90	53/59	90	53/59
100% (severes)**	72	16/22	75	15/20	71	15/21
 75% (severes) **	95	21/22	100	20/20	95	20/21

*Balls et al. (1995)

**Scores for fluorescein retention and corneal swelling were not provided for one severe irritant/corrosive (30% trichloroacetic acid), which was therefore classified based on results from only 3 laboratories



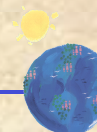
ICE Interlaboratory %CV Values*

		%CV		
		FR	CO	CS
Total (59 Substances)	Mean	38.8	46.8	77.2
	Median	35.6	37.1	74.5
	Range	0-158.7	0-158.7	30.8-159.4
GHS Category 1 (22 Substances)	Mean	29.9	34.2	72.4
	Median	23.0	25.0	69.5
	Range	0-158.7	0-118.6	32.2-132.2

*Balls et al. (1995)

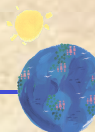
CO: Corneal opacity; CS: Corneal swelling; CV: standard deviation/mean; %CV: Coefficient of variation, expressed as a percentage; FR: Fluorescein retention

Interlaboratory %CV values based on results from four laboratories



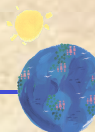
Limitations of ICE Reliability Analysis

- **Intralaboratory reliability unknown due to lack of published data**
- **Interlaboratory reproducibility based on only one study (4 laboratories, 59 substances)**



Draft ICE BRD Proposals (1)

- A proposed ICE version, which evaluates corneal opacity, corneal swelling, fluorescein retention, and morphological effects
- A proposed standardized protocol
 - Protocol based on method of TNO Nutrition and Food Research (INVITTOX 2004)
 - Only significant difference is inclusion of additional eyes for negative controls, a concurrent positive control, and, when appropriate, benchmark controls



Draft ICE BRD Proposals (2)

- **Proposed additional optimization studies, including:**
 - Retrospective analysis of decision criteria used to identify corrosives and severe irritants
 - An evaluation of the potential causes of the greater level of interlaboratory variability for the corneal swelling endpoint
 - Additional evaluation of possible increased interlaboratory variability for specific chemical classes appearing more variable, based on the small numbers of representative substances in this evaluation (i.e., alcohols, acetates/esters, cationic surfactants)
 - Determining the feasibility of introducing quantitative measurement for corneal opacity
 - Determining the utility of histopathology and when it should be included.
- **Once optimized, additional validation studies to further characterize accuracy and reliability of the optimized method**

